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Low Temperature Effect on Selective Fertilization by Pollen Mixtures of Wild and Cultivated Tomato Species

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Summary. In vitro pollen germination of cultivated tomato, *Lycopersicon esculentum* Mill., is inhibited by an ambient temperature of 5°C, more so than pollen from a Peruvian ecotype of *Lycopersicon hirsutum* Humb. & Bonpl. originating from an altitude of 3200 m. The frequency of *L. hirsutum* gametes contributing to hybrid zygote formation is more than doubled when controlled fertilizations with pollen mixtures of the two species occurs at 12/6°C as compared to crosses with the same mixtures at 24/19°C. The results suggest that differential selection at the gametophytic level occurs in response to low temperature regimes. To our knowledge this is the first time in higher plants that alteration of an environmental factor has been demonstrated to change selection values of male gametophytes in a fashion predicted by the ecology of the parental sporophytes.

Key Words: Low temperature adaptation – *Lycopersicon* – Pollen mixtures – Selective fertilization

Introduction

The cultivated tomato *L. esculentum* Mill. and ecotypes of the related green fruited species *L. hirsutum* Humb & Bonpl. found in the Peruvian Andes at elevations up to 3200 m (Rick et al. 1979) differ in their ability to tolerate low temperature stress conditions (Patterson et al. 1978). High altitude *hirsutum* demonstrate more tolerance to low temperature with respect to a number of physiological characters including seed germination, seedling survival, chlorophyll development (Patterson et al. 1978), rate of protoplasmic streaming (Patterson and Graham 1977), and amino acid uptake by leaf tissue (Paull et al. 1979). Moreover, the degree of chilling resistance for most of these characters is correlated with the altitude of origin of *hirsutum* ecotypes. *L. esculentum* reacts to chilling in a manner similar to *hirsutum* ecotypes found near sea level, showing poor tolerance.

L. hirsutum from high elevations can complete its life cycle in an environment where the minimum daily temperatures often drop below 6°C (Johnson 1976). This implies that the haploid pollen grains can germinate, penetrate the stylar tissue and complete fertilization in a cold environment. The present study was initiated to determine if pollen grains of high altitude *hirsutum* differ in their ability to germinate and effect fertilization at low temperature in comparison to chilling-sensitive *esculentum*.

Materials and Methods

In vitro Pollen Germination

Pollen was collected a day after anthesis from *L. esculentum* cv. 'T5', a fresh market variety from California, and *L. hirsutum* (LA 1777) plants which originated from a wild population growing in Peru at elevations of 3200 m (Rick et al. 1979). Plants were grown in the greenhouse at temperature regimes of 24/18°C (day/night). Pollen was collected from both genotypes and germinated on medium containing 18% sucrose, 1% agar and 0.015% boric acid. The medium was poured into petri dishes as recommended by Pfahler (1967). Pollen of each genotype was plated on duplicate petri dishes and incubated at both 15°C and 5°C in the dark. The temperature of 15°C was selected because preliminary studies indicated that higher temperatures resulted in a large percentage of ruptured pollen grains. Plates were scored for percent germination after 8 hr at 15°C and 100 hr in the 5°C treatment. Germination was scored using a microscope at X 100 magnification, and a grain was classified as germinated if the pollen tube length was at least half the pollen diameter. A total of 100 grains per petri dish were sampled per treatment by counting fields of 20-40 well-separated pollen grains. Experiments were repeated on three separate occasions.

Pollen Mixtures and Selective Fertilization

Three different pollen mixtures were prepared from equal volumes of pollen harvested from flowers of *L. hirsutum* (LA 1777) and *L.*

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esculentum cv. 'T5' and thoroughly mixed with a vortex mixer. Greenhouse-grown plants of a male-sterile *L. esculentum* (ms 10^{35}) line were transferred to growth chambers and served as pistillate parents for the controlled pollinations with the mixtures. One growth chamber was set at 6 hr cycles of 24°C (light; 40 μE $m^{-2} s^{-1}$), 19°C (dark). The second chamber was on an identical time cycle and light intensity but maintained at 12°C (light), 6°C (dark). Preliminary experiments indicated that constant temperatures of 7°C for 96 hr resulted in no fruit set. Low light intensities were selected to minimize temperature gradients in the growth chamber. The 3 pollen mixtures were applied to virgin stigmas at the beginning of the dark period. Four days following pollination the plants were removed from the growth chambers to the greenhouse. Pollinated stigmas were excised at midlength with a razor blade to prevent late fertilizations under the higher greenhouse temperatures.

The two species differ in alleles at *Adh-1*, a locus on chromosome 4 responsible for alcohol dehydrogenase activity in mature seeds (Tanksley 1979). Progeny seed from the mixed pollen crosses were individually assayed for *Adh-1* in order to determine the frequency of egg cells which were fertilized by the different male gametes. The electrophoretic analysis was conducted on seeds from 2 to 4 fruits in each treatment. Enzyme extraction, starch gel electrophoresis conditions and activity staining were as described by Tanksley (1979).

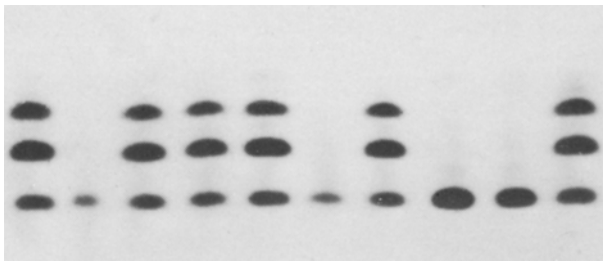

Fig. 1. Photograph of starch gel stained for alcohol dehydrogenase activity. Each lane represents an extract of a single progeny seed from the mixed pollination experiment. The lanes with three dark bands are heterozygote (an *esculentum* ovule fertilized by a *hirsutum* pollen grain) and those with one major band are homozygotes (an *esculentum* ovule fertilized by an *esculentum* pollen grain)

Results and Discussion

The ratio of percent pollen germination in vitro at 5°C to that at 15°C was used to represent the effect of temperature on pollen germination. Analysis of the difference between the ratios for the two *Lycopersicon* species indicates that in vitro germination of *esculentum* pollen is significantly more inhibited by cold temperature (5°C) than is pollen of the high altitude *hirsutum* (Table 1).

In three separate experiments, mixtures of *esculentum* and *hirsutum* pollen were applied to stigmas of the male-sterile *esculentum* line maintained in growth chambers at either 24/19°C or 12/6°C temperature regimes. The existing electrophoretic variation between the two species for *Adh-1* which is expressed in seeds provided an unequivocal means to determine the source of the male gamete contributing to zygote formation in such crosses. Individual seeds were scored for homozygosity or heterozygosity at the *Adh-1* locus (Fig. 1). The proportion of *hirsutum* progeny seed (heterozygous individuals) was more than doubled at 12/6°C compared to 24/19°C in all the mixed pollen crosses (Table 2).

These results suggest that pollen grains of *hirsutum* are favored during the reproductive process at low temperatures. The selection detected in these experiments could have occurred at the following stages: 1) pollen germination, 2) pollen tube growth through the stylar tissue, 3) fertilization, or 4) during early zygotic stages. The first

Table 1. Differential effect of temperature on germination ratio of *L. hirustum* and *L. esculentum* pollen in vitro

	L. esculentum			*L. hirsutum*				
	15°C	5°C		15°C	5°C			
Expt. no.	% Germ.	% Germ.		% Germ.	% Germ.			
	A	B	B/A	C	D	D/C	D/C-B/A	Sd[a]
1	41.5	0.0	0.0	76.5	27.0	0.353	0.353[b]	0.043
2	51.5	5.5	0.107	73.0	16.5	0.226	0.119[c]	0.049
3	49.0	0.5	0.010	50.0	8.0	0.160	0.150[b]	0.041

[a] $Sd = \sqrt{\frac{1}{n}\left(\frac{B(1-B)}{A^2} + \frac{B^2(1-A)}{A^3} + \frac{D(1-D)}{C^2} + \frac{D^2(1-C)}{C^3}\right)}$

where n (sample size) = 200 for all estimates and A, B, C, D expressed as fractions

[b] Significant difference at 0.001 level

[c] Significant difference at 0.05 level

Table 2. Percent progeny derived from *L. hirsitum* in mixed pollen crosses at 2 temperature regimes

Pollen mixture	24 – 19°C		12 – 6°C			
	L. hirsutum: L. esculentum (Progeny ratio)	% *L. hirsutum* (A)	*L. hirsutum: L. esculentum* (Progeny ratio)	% *L. hirsutum* (B)	B-A	Sd[a]
1	45:84	34.9	82:17	82.8	47.9[b]	5.7
2	22:43	33.8	43:5	89.6	55.7[b]	7.3
3	19:24	44.2	28:0	100.0	55.8[b]	7.6

[a] $Sd = \sqrt{\frac{A(1-A)}{n_1} + \frac{B(1-B)}{n_2}}$

where n_1 and n_2 are respective sample sizes for estimates at different temperature regimes and A, B are expressed as fractions

[b] Significant difference at 0.001 level

three stages involve interaction of the male gamete with the pistil and can be considered as selection at the gametophytic level resulting from competition between pollen grains for successful fertilization of the ovules. The latter can occur if zygotes produced by *esculentum* pollen were selectively eliminated due to the low temperature regime in the growth chamber. Several studies bear on the possibility of zygote elimination. In vivo pollen germination experiments in tomato have demonstrated that pollen which had developed at 18°C, took, at 10°C, 84 hr to produce tubes that penetrated the ovary (Charles and Harris 1972). Under these conditions seed set was observed. In the mixed pollen experiments described here, plants were exposed to temperature regimes of 12/6°C for 96 hr, suggesting that zygote elimination is not a major factor affecting the results. The in vitro germination studies are consistent with the suggestion that selective fertilization in the low temperature crosses resulted from selection at the gametophytic level.

Pollen grains form and mature in the anthers. During microsporogenesis, diploid tapetal tissue (the parental sporophyte) and the pollen cytoplasm (the haploid gametophyte) interact to determine the behavior of the mature pollen grain. The present study provides evidence for the low temperature competitive ability of high altitude *L. hirsutum* pollen in the pistils of an *esculentum* variety. We do not differentiate between the possibilities that the cold tolerance of the pollen is due to gene products of the diploid sporophyte which might precondition the gamete against the environmental stress or due to genetic information transcribed in the haploid pollen.

Recently Mulcahy (1979) hypothesized that the adaptive success of angiosperms may have arisen in part through intensification of microgametophytic selection. One of the assumptions is an overlapping model where certain genes which are expressed in the gametophytic stage may also function in the sporophyte and hence selecting these genes in the haploid phase could have a positive effect on the success of the diploid phase. We have shown that a *hirsutum* ecotype which has been demonstrated to be chilling tolerant in the sporophytic stage (see Introduction) also has greater tolerance to low temperature in the gametophytic stage. It remains to be determined whether the haploid genome is responsible for the pollen behavior, and if so, whether the genetic basis of chilling tolerance overlaps in the gametophytic and sporophytic stages. Projects have been initiated in our laboratory aimed at providing answers to both of these questions.

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